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# Effect of a granulovirus on mortality and dispersal of potato tuber worm (Lepidoptera: Gelechiidae) in refrigerated storage warehouse conditions

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#### RESEARCH ARTICLE

## Effect of a granulovirus on mortality and dispersal of potato tuber worm (Lepidoptera: Gelechiidae) in refrigerated storage warehouse conditions

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Potato tuber worm (PTW), Phthorimaea operculella (Zeller), is a world-wide pest of potato. In rustic stores, PTW larvae can infest 100% of stored tubers. Treatment of tubers in rustic stores with the PTW granulovirus (PoGV) has been demonstrated to protect stored tubers. This is the first study to show the effects of PoGV for protection of tubers stored in refrigerated warehouse conditions. Tubers were treated by dipping in aqueous suspensions of PoGV or water. An estimated 0.0819 larval equivalents of virus or  $1.88 \times 10^9$  viral occlusion bodies were deposited on each kilogram of tubers. They were held at 16°C for 11 days before lowering the temperature by 0.5°C per day until 10°C was reached. The tubers were stored at this temperature for 53 days. Mean numbers of infested tubers at the end of the assay was affected by both pre-infestation rate and virus treatment. Mean numbers of infested tubers in the control treatment was 3 tubers per chamber higher than in the virus treatment providing strong evidence that PoGV controlled larvae and minimized spread into un-infested tubers. Of the larvae that were retrieved in virus-treated infested tubers, the mean mortality was 87% compared to 37% in controls.

**Keywords:** potato tuber worm; *Phthorimaea operculella*; granulovirus; tuber storage; *Solanum tuberosum* 

#### Introduction

The potato tuber worm (PTW), *Phthorimaea operculella* (Zeller), is a world-wide pest of potato and other solanaceous vegetables (Radcliffe 1982; Raman 1994; Kroschel and Lacey 2008). In tropical and subtropical agroecosystems, it is considered the most damaging insect pest of potato (Kroschel and Lacey 2008). Larvae mine both leaves and tubers in the field and tubers in storage making the pest difficult to control. It can also be a serious pest in temperate zones on an intermittent basis but long cold winters generally restrict its development and reduce its severity as a pest (Rondon, Debano, Clough, Hamm, and Jensen 2008; Sporleder, Simon, Juarez, and Kroschel 2008). PTW is especially injurious in rustic (non-refrigerated) stores of tubers under tropical conditions (Raman, Booth, and Palacios 1987; von Arx, Goueder, Cheikh, and Temime 1987; Kroschel and Lacey 2008) (Figure 1A). A wide variety of broad spectrum insecticides are employed in potato production including, in some cases,

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Figure 1. (A) Rustic storage of potato tubers near Huancayo, Peru (photo by L. Lacey). (B) Typical refrigerated storage of tubers in the Pacific Northwest of the USA. Quantities of 2270 to 11,343 metric tons of tubers are stored in such warehouses. The most commonly used size stores 6800 metric tons of tubers (photo from Washington State Potato Commission archives. Used with permission).

treatment of tubers (Kroschel and Koch 1996). Their effects on applicators, the food supply and environment have encouraged development of an integrated approach for PTW control (von Arx et al. 1987; Fuglie, Salah, Essamet, Temime, and Rahmouni 1993; Kroschel 1995; Keller 2003; Chandel, Chandla, and Garg 2008; Herman 2008; Horne and Page 2008). Non-chemical methods of control in IPM of PTW includes a variety of soft interventions (Raman et al. 1987; Das, Magallona, Raman, and Adalla 1992; Farrag 1998; Jensen 2006; Chandel et al. 2008; Clough, Debano, and Hamm 2008; Kroschel and Lacey 2008). Among these, the granulovirus of PTW (PoGV) has been shown to provide effective control under field and storage conditions (Alcázar, Cervantes, and Raman 1992; Das et al. 1992; Kroschel, Fritsch, and Huber 1996; Zeddam, Vasquez Soberon, Vargas Ramos, and Lagnaoui 2003; Arthurs, Lacey, and de la Rosa 2008a; Arthurs, Lacey, Pruneda, and Rondon 2008b; Sporleder and Kroschel 2008; Lacey and Kroschel 2009).

Tubers are subject to infestation before harvest, when fissures in potato hills allow ingress of and oviposition by female PTW and after harvest. Tubers infested by first instars are barely detectable. Subsequently, further propagation of PTW may take place, especially in non-refrigerated storage where the damage to tubers can reach 100% within a few months if they are left untreated. In developed countries, potato tubers are stored in large refrigerated (3–13°C) warehouses (Figure 1B). Temperature is dependant on the purpose and cultivar of the tubers (Knowles and Plissey 2007). Development of PTW larvae slows considerably at cooler temperatures and at less than 10°C, ceases completely (Sporleder, Kroschel, Gutierrez Quispe, and Lagnaoui 2004). In large warehouses, temperatures are reduced slowly to facilitate curing and wound healing (Knowles and Plissey 2007). The time required to reach storage temperature could enable significant PTW larval development. Currently no insecticides are registered for postharvest use on potato tubers in North America. The objective of our research was to evaluate PoGV for control of PTW in conditions that simulate refrigerated commercial storage.

#### Materials and methods

#### Tuber worm colony

The PTW used in our study were originally collected in 2004 from a field population near Hermiston, Oregon. The rearing procedure to maintain the colony and produce virus was described by CIP (1992) and modified by Arthurs et al. (2008b). Briefly, rearing was done in 7.6 L Tupperware<sup>®</sup> containers in tubers that were infested with eggs that were within 24 h of eclosion. Sand was placed on the bottom of containers as a pupation substrate. The infested tubers were incubated for 16 days at 27°C, 25% RH and 16 h L:8 h D, after which pupae were separated from the sand using a 1-mm sieve. The cocoons were then dissolved in 1.25% NaClO, rinsed and placed in 0.47-L oviposition cups (approx. 200 pupae/cup) fitted with polyester knit tricot interfacing fabric (Staple Sewing Aids Corporation, Garfield, NJ) covers and 9-cm diameter filter papers placed on top as an oviposition substrate. Emerging moths were fed 15% sugar solution on cotton wicks. Eggs were collected daily for 5 days and filter papers were replaced between collections. These were used for colony maintenance, larvae for experiments, and virus production.

#### Virus production and preparation

PoGV was produced in PTW larvae essentially using the same procedures as those for rearing. The PoGV isolate used in our studies originated from a powder formulation produced by the International Potato Center in Lima, Peru, re-isolated from *Tecia solanivorax* Povolny by Birgit Schaub (CIP, Quito, Ecuador) and imported under permit #29878. Eggs on filter papers and tubers were dipped in viral suspensions made with one virus-infected fourth instar per ml of de-ionized water (=larval equivalent [LE]/mL). After drying, they were placed in rearing containers and incubated as described above. Infected larvae exited the tubers 21-28 days after incubation. They were then frozen until used for the storage studies. Quantification of occlusion bodies (OB) by Arthurs et al. (2008b) in our laboratory estimated that each LE contained an average of  $2.3 \times 10^{10}$  OB.

Infected larvae were prepared for treatments by placing 20,000 larvae and 1 L of deionized water in an Osterizer<sup>®</sup> blender and blending for 2 min with 10-s pulses to avoid overheating. Another liter of water was used to rinse the blender. The rinse water was combined with the triturated larvae and the suspension was then vacuum filtered through three layers of organdy using a Nalgene<sup>™</sup> disposable filter with the filter removed to trap head capsules and other large insect parts. The two liters of PoGV suspension were divided into 50 lots, each containing 400 LE of virus.

#### Evaluation of PoGV for PTW control in refrigerated storage

Tubers used to establish an initial infestation were purchased from a grocery store, washed in tap water and dried. One hundred and sixty tubers were individually infested with five neonate larvae in 0.5 L ventilated cups and incubated for 5 days at  $25^{\circ}$ C. Cabinets with individual storage chambers ( $76.2 \times 61.0 \times 30.5$  cm) within two temperature-controlled rooms were used for tuber storage (Figure 2A, B). Each chamber was ventilated through screened intake and exhaust openings. Air flow was maintained by creating negative pressure in the chambers with a fan. The temperature



Figure 2. (A) Storage compartments. Air flow is continuous through screened anterior and posterior ports. (B) Example of a storage chamber used for studies on the effectiveness of PoGV to prevent spread of potato tuber worm larvae from infested tubers to nearby tubers. (C) Treatment and drying of tubers (photos by H. Headrick).

in each room was recorded with a Hobo Pro<sup>™</sup> Series data logger (Onset Computer, Pocasset, MA, USA). Tubers used in the experiments were harvested in Franklin County, WA, an area free of PTW in 2008. Each of 35 storage chambers received 50 tubers for a total of 1750 tubers. Each of the treatments and controls were replicated 5 times. Ten of the chambers included 3 infested tubers; 10 included 5 infested tubers; and 10 included 8 infested tubers, to produce initial infestation rates of 6, 10, and 16%, respectively. Five chambers had no infested tubers to verify that the Franklin County tubers were PTW-free. All PoGV treatments were made in 40 L of water in 68.1-L plastic bins to prevent overflow when 10 kg of tubers were dipped (Figure 2C). Viral suspensions were prepared by adding concentrates of PoGV described above into 40 L of tap water into each of two bins resulting in 0.01 LE/ml. Twenty milliliters Silwet L-77<sup>®</sup> (=0.05%) were added to the bins as a wetting agent. A random sample of 10 medium sized tubers (mean 144 g each) was used to determine the average amount of water retained by each tuber by subtracting dry weight from wet weight of each. An average of 1.18 mL/tuber was retained, the equivalent of 0.0118 LE/tuber or 0.0819 LE/kg  $(1.88 \times 10^9 \text{ OBs/kg})$ .

Ten kilograms of tubers were dipped at a time into the virus suspensions using plastic baskets (Figure 2C). Fifty kilograms of tubers were treated before changing the suspensions in each bin. Controls were dipped in tap water with 0.05% Silwet. Treated

tubers were placed on racks and dried using electric fans (Figure 2C) before placing them in the storage chambers (Figure 2B). Infested tubers were marked with an 'X' using a Sharpie<sup>®</sup> pen. Those touching infested tubers were marked with a slash and tubers not in contact with infested tubers were left un-marked (Figure 2B). Four chambers were included in each cabinet. The seven treatments were randomly assigned to the chambers and rooms. The initial temperature in the rooms was maintained at 16°C for 11 days (10/24/08 to 11/03/08) before gradually lowering it by 0.5°C per day until 10°C was reached. The tubers were stored at this temperature for 53 days before removing them on January 6/09 for dissection to determine rate of infestation. An infested tuber was defined as one with any signs of entry (frass on the tuber surface), presence of one or more galleries and larvae. Living and dead larvae were separately counted for each treatment and controls, level of pre-infestation and location within the tuber pile (pre-infested, touching and non-touching).

#### Statistical analysis

Effects of pre-infestation levels (3, 5, or 8 pre-infested tubers) and virus treatment (virus vs. control) on numbers of infested tubers or larval mortality were assessed using factorial analysis of variance (ANOVA). The analyses were done using PROC MIXED in SAS (SAS Institute 2002). In the event of significant interaction between factors, we compared simple effects means using the SLICE command.

#### Results

To confirm that numbers of un-infested tubers in contact with pre-infested tubers at the beginning of the experiment were similar between virus and control treatments, we conducted a  $3 \times 2$  (pre-infestation level × treatment) analysis of variance (Table 1:

Table 1. Treatments and resulting mean number of potato tuber worm infested tubers per chamber after 53 days of storage at  $10^{\circ}$ C.

	Mean number of tubers per chamber					
·	Pre-si	torage	Post-storage			
Treatments/no. pre-infested tubers/chamber	Number touching tubers	Number non-touching tubers	Number touching infested tubers	Number non-touching infested tubers		
Control/3 Control/5 Control/8 Control mean	$20.8 \pm 1.93$ $33.6 \pm 3.93$ $36.0 \pm 1.30$ $30.1 \pm 4.72$	$25.4 \pm 2.02$ $12.4 \pm 3.57$ $6.0 \pm 1.30$ $14.6 \pm 5.71$	$\begin{array}{c} 1.6 \pm 0.51 \\ 5.0 \pm 1.67 \\ 5.8 \pm 0.66 \\ 4.1 \pm 0.46 \end{array}$	$0.4 \pm 0.25$ $1.4 \pm 0.98$ $0.8 \pm 0.37$ $0.9 \pm 0.46$		
PoGV-treated/3 PoGV-treated/5 PoGV-treated/8 PoGV mean	$13.6 \pm 1.81$ $32.4 \pm 3.17$ $34.6 \pm 2.32$ $26.9 \pm 6.66$	$34.8 \pm 2.15$ $12.6 \pm 3.17$ $7.6 \pm 1.33$ $18.3 \pm 8.36$	$0.8 \pm 0.37$ $1.4 \pm 0.60$ $2.2 \pm 1.24$ $1.5 \pm 0.46$	$0.2 \pm 0.20$ $1.2 \pm 0.97$ $0.2 \pm 0.20$ $0.5 \pm 0.46$		

<sup>&</sup>lt;sup>1</sup>Each treatment was replicated five times using 50 tubers in each storage chamber. Virus application estimated at 0.083 larval equivalents per kg of tubers.

left-most column). Although there is a suggestion that the numbers of uninfested tubers in contact with pre-infested tubers was larger for the control treatment (mean = 20.8 tubers; Table 1) than virus treatment (mean = 13.6 tubers; Table 1) at the pre-infestation rate of 3 tubers per chamber, neither the interaction term ( $F_{2,24} = 0.9$ , P = 0.43) nor the treatment main effects term ( $F_{1,24} = 24$ , P = 0.13) were significant. Thus, we conclude that virus and control treatments were similar whether un-infested tubers were in contact with pre-infested tubers or not, and that the effects of the virus on final rates of tuber infestation (see below) were truly due to the virus and not to differences between control and virus treatments in pre-assay conditions. As expected, numbers of un-infested tubers in contact with pre-infested tubers increased with pre-infestation rates from 3 to 8 tubers per chamber (Table 1, left-most column;  $F_{2,24} = 29.4$ , P < 0.0001). There were no signs of infestation in the five chambers having no pre-infested tubers indicating that the tubers harvested in Franklin County were PTW-free.

Total numbers of infested tubers per chamber at the end of the assay (Figure 3) was affected by both pre-infestation rate ( $F_{2,24} = 6.5$ , P = 0.006) and virus treatment ( $F_{1,24} = 14.6$ , P = 0.0008); the interaction term was non-significant ( $F_{2,24} = 1.7$ , P = 0.21). Mean numbers of infested tubers in the control treatment was 3 tubers per chamber higher than in the virus treatment, when data are averaged over pre-infestation rates (Figure 3; right-most symbols), which is strong evidence that the virus provided control of larvae.

The results summarized in Figure 3 were then re-examined by first separating the overall infestation data (Figure 3) into two categories (Table 1: right-most two columns): infestation of tubers that were in contact with pre-infested tubers ('touching'); and infestation of tubers that were not in contact with pre-infested tubers ('non-touching'). Thus, this second analysis examined whether infestation rates of tubers depended upon whether the tubers were in contact with pre-infested tubers,

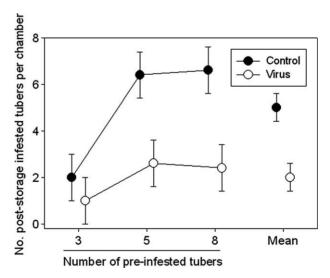


Figure 3. Mean number of PTW-infested tubers per storage chamber by treatment and infestation rate after 53 days at 10°C. Mean numbers of infested tubers when data are averaged over pre-infestation rates (right-most symbols).

and then assessed whether the virus affected this tendency. We used a three-factor (treatment × pre-infestation rate × tuber contact category) ANOVA to examine these questions. The treatment by contact category interaction was significant ( $F_{1.48} = 6.4$ , P = 0.015), thus simple effects means were examined. Tests on simple effects showed that infestation rates were higher in touching tubers (Table 1: mean = 4.13 tubers per chamber, averaged over pre-infestation rate) than non-touching tubers (mean =0.87tubers per chamber) in the control treatment ( $F_{1.48} = 24.9$ , P < 0.0001) but not in the virus treatment (means of 1.47 and 0.53 tubers per chamber for touching and nontouching tubers, respectively;  $F_{1.48} = 2.0$ , P = 0.16). A comparison of virus vs. control treatments indicated that infestation rates were higher in the control treatment (Table 1: mean = 4.13 tubers per chamber) than virus treatment (1.47 tubers) for touching tubers ( $F_{1.48} = 16.6$ , P < 0.0002); treatment did not affect infestation rates of nontouching tubers (Table 1: 0.87 vs. 0.53 tubers per chamber; P = 0.61). These results indicate that treatment with virus prevented most movement by larvae into adjacent ('touching') tubers. Of the larvae retrieved from virus-treated 'touching' tubers, the mean mortality rate was 87% compared to 37% in controls.

Tests on whether numbers of live or dead larvae recovered from pre-infested tubers differed between treatments or among pre-infestation rates were assessed using factorial ANOVA (Table 2; left two columns). The treatment  $\times$  status (live vs. dead) interaction was significant ( $F_{1,48} = 6.0$ , P = 0.018), thus we conducted tests on simple effects means. Averaging across infestation rates in pre-storage and virus-treated tubers, there were significantly ( $F_{1,48} = 13.9$ , P < 0.0005) more dead larvae (Table 2: mean = 4.40 larvae) than live larvae (mean = 0.60 larvae), whereas there was no significant difference between numbers of live and dead larvae in the control treatment (Table 2: 2.27 vs. 2.53 larvae;  $F_{1,48} = 0.1$ , P = 0.79). These results suggest there was good penetration of PTW galleries by the viral suspensions.

Table 2. Treatments and resulting number of live and dead larvae per tuber after 53 days of storage at 10°C.<sup>1</sup>

	Mean num	ean number of live and dead larvae ± SEM per chamber after storage					
	Pre-infested tubers		Infested touching tubers		Infested non-touching tubers		
Treatments/no. pre-infested tubers/chamber	Live	Dead	Live	Dead	Live	Dead	
Control/3 Control/5 Control/8 Control mean	$1.6 \pm 0.93$ $1.8 \pm 0.66$ $3.4 \pm 0.80$ $2.3 \pm 0.72$	$0.6 \pm 0.60$ $4.6 \pm 1.33$ $2.4 \pm 0.89$ $2.5 \pm 0.72$	$0.8 \pm 0.49$ $3.2 \pm 1.59$ $3.0 \pm 1.22$ $2.3 \pm 0.77$	$0.8 \pm 0.58$ $0.6 \pm 0.24$ $1.8 \pm 0.66$ $1.1 \pm 0.37$	$0 \\ 0.6 \pm 0.60 \\ 0.2 \pm 0.20 \\ 0.3 \pm 0.18$	$0.4 \pm 0.24 \\ 0.6 \pm 0.60 \\ 0.4 \pm 0.24 \\ 0.5 \pm 0.07$	
PoGV-treated/3 PoGV-treated/5 PoGV-treated/8 PoGV mean	$\begin{array}{c} 1.2 \pm 0.80 \\ 0.4 \pm 0.40 \\ 0.2 \pm 0.20 \\ 0.6 \pm 0.72 \end{array}$	$4.2 \pm 2.06$ $2.4 \pm 1.44$ $6.6 \pm 2.54$ $4.4 \pm 0.72$	$0\\0.2\pm0.20\\0\\0.1\pm0.07$	$0 \\ 0.3 \pm 0.22 \\ 1.2 \pm 0.80 \\ 0.5 \pm 0.37$	$0.2 \pm 0.20$ $0.2 \pm 0.20$ $0.2 \pm 0.20$ $0.2 \pm 0.0$	0 0 0 0	

<sup>&</sup>lt;sup>1</sup>See footnote in Table 1.

#### Discussion

The application of PoGV to stored tubers provides a high degree of protection under rustic conditions (Alcázar et al. 1992; CIP 1992; Zeddam et al. 2003; Chandel et al. 2008). The results of our research on PoGV for protection of stored tubers in refrigerated conditions demonstrate the effectiveness of the virus in decreasing the spread of larvae from infested tubers into those nearby. Although a small number of larvae were found in infested PoGV-treated touching tubers, the majority of these (87%) were dead. In the different infestation levels of 3, 5 and 8 tubers, a total of 15, 25 and 40 larvae were, respectively, introduced into the chambers. However, only a small number of live and dead larvae were recovered resulting in a large number of larvae that were not found. For example, the mean number of larvae, both alive and dead, found in the control chambers receiving 8 infested tubers was 11.2 or 28% of the larvae used for infestation. The larvae used for infestation had adequate incubation at 16–25°C to develop to second or third instars. However, if larvae exited from tubers, but did not re-enter another, they could desiccate sufficiently after 74 days in the chambers to become undetectable. It is also possible that some mortality of neonate larvae occurred briefly after the infestation of tubers.

The conditions under which our tests were conducted are similar to those in commercial stores for Russett Burbank tubers (i.e., gradual decrease in temperature from ambient to the long-term storage temperature with constant air circulation). Initial incubation at 25°C after exposing tubers to neonate larvae increased the likelihood that larvae underwent some development before tests began at an ambient 16°C. The 2-week time interval between infestation and lowering storage temperature below ambient would enable additional development and survival of larvae in untreated tubers (Sporleder et al. 2004). However, the effect of holding tubers at 10°C for 53 days had a pronounced effect on the survival of control larvae. The movement of larvae from one tuber to another in both virus treatments and controls probably took place before temperatures declined much below 16°C.

Eggs and initial entries by neonate larvae are virtually undetectable in tubers at the time they are placed into storage. Sporleder, Rodriguez Cauti, Huber, and Kroschel (2007) and Arthurs et al. (2008a) observed that young larvae, especially neonates, were considerably more susceptible to PoGV than older instars. Concentrations of PoGV as low as 0.0065 LE/kg of tubers can produce 100% mortality in neonate larvae (Arthurs et al. 2008a). Larvae hatching from eggs on the surface of tubers treated with virus would consume an adequate amount of virus from the surfaces of the eggs and tubers to ensure 100% mortality. Older larvae, especially those inside of tubers are more difficult to control. Arthurs et al. (2008a) observed 88–91% mortality in second and third instars within tubers treated with virus suspensions ranging from 0.025 to 0.4 LE/kg of tubers. The concentration of virus used in our treatments (0.083 LE/kg) fell within the range of concentrations used by Arthurs et al. (2008a). Although some of the parameters differed between the two studies, our results in simulated commercial storage conditions corroborate the laboratory studies of Arthurs et al. (2008a).

Schreiber (2006) demonstrated the effect of constant and variable temperatures (5–16.7°C) and variable infestation rates (10–50%) on dispersal of larvae from infested into un-infested tubers. For example, at 20% initial infestation and 12.2 and 16.7°C resulted in 77% infestation within 15 days. At the 6–16% infestation rates in our research, high mortality in the pre-infested PoGV-treated tubers resulted in

significantly fewer new infestations. Storage temperatures no doubt contributed to mortality in treated and control larvae. Under typical commercial storage conditions, the initial temperatures may be as high as 24°C before being gradually lowered to 10–12°C (Knowles and Plissey 2007). Although this would enable faster development of larvae and movement to un-infested tubers, PoGV treatment could reduce the number of dispersing larvae. Surviving larvae entering adjacent virus-treated tubers would ingest additional PoGV and increase the likelihood of dying. Unlike field applications, the virus is not exposed to ultra-violet radiation in storage and may protect tubers for several months.

PoGV treatment of tubers by submersion in aqueous virus suspensions in commercial storage will not be feasible due to additional labor requirements, potential for rot and sprouting. If PoGV is to be used in commercial storage for PTW control, a method for surface application such as a fine mist that allows rapid drying will be needed. For PTW control in rustic tuber storage, the virus is or has been commercially produced as powder formulations in Peru, Bolivia, Egypt, and Tunisia using low cost facilities for propagation (Kroschel and Lacey 2008). Based on the need for PTW management during the vegetative growth of potato, the potential of PoGV application as a method to slow development of resistance to conventional insecticides, its safety and potential for incorporation into IPM systems with minimal impact on beneficial non-target organisms, further commercial development is warranted.

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#### References

- Alcázar, J., Cervantes, M., and Raman, K.V. (1992), 'Efectividad de un Virus Granulosis Formulado en Polvo para Controlar *Phthorimaea* en Papa Almacenada', *Revista Peruana de Entomología*, 35, 113–116.
- Arthurs, S.P., Lacey, L.A., and de la Rosa, F. (2008a), 'Evaluation of a Granulovirus (PoGV) and *Bacillus thuringiensis* subsp. *kurstaki* for Control of the Potato Tuberworm in Stored Tubers', *Journal of Economic Entomology*, 101, 1540–1546.
- Arthurs, S.P., Lacey, L.A., Pruneda, J.N., and Rondon, S. (2008b), 'Semi-Field Evaluation of a Granulovirus and *Bacillus thuringiensis* ssp. *kurstaki* for Season-Long Control of the Potato Tuber Moth, *Phthorimaea operculella*', *Entomologia Experimentalis et Applicata*, 129, 276–285.
- Chandel, R., Chandla, V., and Garg, I. (2008), 'Integrated Pest Management of Potato Tuber Moth in India', in *Integrated Pest Management for the Potato Tuber Moth, Phthorimaea operculella Zeller a Potato Pest of Global Importance*, eds. J. Kroschel and L. Lacey, Tropical Agriculture 20, Advances in Crop Research 10, Weikersheim, Germany: Margraf Publishers, pp. 127–138.
- CIP (1992), 'Biological Control of Potato Tuber Moth Using *Phthorimaea* baculovirus', *CIP Training Bulletin* 2, Lima, Peru: International Potato Center, 27 pp.

- Clough, G.H., Debano, S.J., and Hamm, P.B. (2008), 'Reducing Potato Tuber Moth Damage with Cultural Practices and Pesticide Treatments', in *Integrated Pest Management for the Potato Tuber Moth, Phthorimaea operculella Zeller a Potato Pest of Global Importance*, eds. J. Kroschel and L. Lacey, Tropical Agriculture 20, Advances in Crop Research 10, Weikersheim, Germany: Margraf Publishers, pp. 101–109.
- Das, G.P., Magallona, E.D., Raman, K.V., and Adalla, C.B. (1992), 'Effects of Different Components of IPM in the Management of the Potato Tuber Moth in Storage', Agriculture Ecosystem and Environment, 41, 321–325.
- Farrag, R.M. (1998), 'Control of the Potato Tuber Moth, *Phthorimaea operculella* Zeller (Lepidoptera Gelechiidae) at Storage', *Egyptian Journal of Agricultural Research*, 76, 947–952.
- Fuglie, K., Salah, H.B., Essamet, M., Temime, A.B., and Rahmouni, A. (1993), 'The Development and Adoption of Integrated Pest Management and the Potato Tuber Moth, *Phthorimaea operculella* (Zeller) in Tunisia', *Insect Science and its Application*, 14, 501–509.
- Herman, J.B. (2008), 'Integrated Pest Management of Potato Tuber Moth in New Zealand', in *Integrated Pest Management for the Potato Tuber Moth, Phthorimaea operculella Zeller a Potato Pest of Global Importance*, eds. J. Kroschel and L. Lacey, Tropical Agriculture 20, Advances in Crop Research 10, Weikersheim, Germany: Margraf Publishers, pp. 119–126.
- Horne, P., and Page, J. (2008), 'Integrated Pest Management Dealing with Potato Tuber Moth and All Other Pests in Australian Potato Crops', in *Integrated Pest Management for the Potato Tuber Moth, Phthorimaea operculella Zeller a Potato Pest of Global Importance*, eds. J. Kroschel and L. Lacey, Tropical Agriculture 20, Advances in Crop Research 10, Weikersheim, Germany: Margraf Publishers, pp. 111–117.
- Jensen, A. (2006), 'Cull Pile and Waste Potato Management Important for Tuberworm Control', Potato Progress, 6, 4.
- Keller, S. (2003), 'Integrated Pest Management of the Potato Tuber Moth in Cropping Systems of Different Agroecological Zones', Tropical Agriculture 11, Advances in Crop Research 1, Weikersheim, Germany: Margraf Publisher, 156 pp.
- Knowles, N.R., and Plissey, E.S. (2007), 'Maintaining Tuber Health during Harvest, Storage, and Post-Storage Handling', in *Potato Health Management*, 2nd ed. ed. D.A. Johnson, St. Paul, MN, USA: APS Press, pp. 79–99.
- Kroschel, J. (1995), 'Integrated Pest Management in Potato Production in the Republic of Yemen with Special Reference to the Integrated Biological Control of the Potato Tuber Moth (*Phthorimaea operculella* Zeller)', Tropical Agriculture 8, Weikersheim, Germany: Margraf Verlag, 227 pp.
- Kroschel, J., and Koch, W. (1996), 'Studies on the Use of Chemicals, Botanicals and Bacillus thuringiensis in the Management of the Potato Tuber Moth in Potato Stores', Crop Protection, 15, 197–203.
- Kroschel, J., and Lacey, L.A. (eds.), (2008), 'Integrated Pest Management for the Potato Tuber Moth, Phthorimaea operculella (Zeller) a Potato Pest of Global Importance', Tropical Agriculture 20, Advances in Crop Research 10. Weikersheim, Germany: Margraf Publishers, 147 pp.
- Kroschel, J., Fritsch, E., and Huber, J. (1996), 'Biological Control of the Potato Tuber Moth (*Phthorimaea operculella* Zeller) in the Republic of Yemen Using Granulosis Virus: Biochemical Characterization, Pathogenicity and Stability of the Virus', *Biocontrol Science and Technology*, 6, 207–216.
- Lacey, L.A., and Kroschel, J. (2009), 'Microbial Control of the Potato Tuber Moth (Lepidoptera: Gelechiidae)', Fruit, Vegetable, and Cereal Science and Biotechnology 3 (Special Year of the Potato Issue 1), 46–54.
- Radcliffe, E.B. (1982), 'Insect Pests of Potato', Annual Review of Entomology, 27, 173–204.
- Raman, K.V. (1994), 'Potato Pest Management in Developing Countries', in *Advances in Potato Pest Biology and Management*, eds. G.W. Zehnder, M.L. Powelson, R.K. Jansson and K.V. Raman, St Paul, MN, USA: APS Press, pp. 583–596.
- Raman, K.V., Booth, R.H., and Palacios, M. (1987), 'Control of Potato Tuber Moth *Phthorimaea operculella* (Zeller) in Rustic Potato Stores', *Tropical Science*, 27, 175–194.
- Rondon, S.I., Debano, S.J., Clough, G.H., Hamm, P.B., and Jensen, A. (2008), 'Occurrence of the Potato Tuber Moth, in the Columbia Basin of Oregon and Washington', in *Integrated*

- Pest Management for the Potato Tuber Moth, Phthorimaea operculella Zeller a Potato Pest of Global Importance, eds. J. Kroschel and L. Lacey, Tropical Agriculture 20, Advances in Crop Research 10, Weikersheim, Germany: Margraf Publishers.
- SAS Institute (2002), SAS 9.1 for Windows. Cary, NC: USA.
- Schreiber, A. (2006), 'Potato Tuberworm: A Potential New Pest of Potatoes in Washington Storages', *Potato Progress*, 6 (13), 1–2.
- Sporleder, M., and Kroschel. J. (2008), 'The Potato Tuber Moth Granulovirus (*PoGV*): Use, Limitations and Possibilities for Field Applications', in *Integrated Pest Management for the Potato Tuber Moth, Phthorimaea operculella Zeller a Potato Pest of Global Importance*, eds. J. Kroschel and L. Lacey, Tropical Agriculture 20, Advances in Crop Research 10, Weikersheim, Germany: Margraf Publishers, pp. 49–71.
- Sporleder, M., Kroschel, J., Gutierrez Quispe, M., and Lagnaoui, A. (2004), 'A Temperature-Based Simulation Model for the Potato Tuberworm, *Phthorimaea operculella* Zeller (Lepidoptera; Gelechiidae)', *Environmental Entomology*, 33, 477–486.
- Sporleder, M., Rodriguez Cauti, E.M., Huber, J., and Kroschel, J. (2007), 'Susceptibility of Phthorimaea operculella Zeller (Lepidoptera; Gelechiidae) to its Granulovirus PoGV with Larval Age', Agricultural and Forest Entomology, 9, 271–278.
- Sporleder, M., Simon, R., Juarez, H., and Kroschel, J. (2008), 'Regional and Seasonal Forecasting of the Potato Tuber Moth Using a Temperature-Driven Phenology Model Linked with Geographic Information Systems', in *Integrated Pest Management for the Potato Tuber Moth, Phthorimaea operculella Zeller a Potato Pest of Global Importance*, eds. J. Kroschel and L. Lacey, Tropical Agriculture 20, Advances in Crop Research 10, Weikersheim, Germany: Margraf Publishers, pp. 15–30.
- von Arx, R., Goueder, J., Cheikh, M., and Temime, A.B. (1987), 'Integrated Control of Potato Tubermoth *Phthorimaea operculella* (Zeller) in Tunisia', *Insect Science and its Application*, 8, 989–994.
- Zeddam, J.-L., Vasquez Soberon, R.M., Vargas Ramos, Z., and Lagnaoui, A. (2003), 'Producción Viral y Tasas de Aplicación del Granulovirus Usado para el Control Biológico de las Polillas de la Papa *Phthorimaea operculella y Tecia solanivora* (Lepidoptera: Gelechiidae)', *Boletín de Sanidad Vegetal, Plagas*, 29, 659–667.